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HISTOPATHOLOGICAL CHANGES IN THE LIVER OF *ETROPLUS SURATENSIS* (PEARLSPOT) EXPOSED TO SELECTED INSECTICIDE, LAMBDA-CYHALOTHRIN

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ABSTRACT

Histopathological investigations on different tissues of fish are valuable tools for toxicology studies and monitoring water pollutions. Contamination of water by pesticides, either directly or indirectly, can lead to fish kills, reduced fish productivity, or elevated concentrations of undesirable chemicals in edible fish tissue which can affect the health of humans consuming these fish. Fishes were randomly selected for histopathological observations by sampling after 60 days of pesticide exposure. No histopathological effects were observed in the control group. Many alterations in the liver such as necrosis, damaged hepatocytes, lymphatic aggregation, degeneration, rupture of the hepatocytes, haemorrhage, coagulative necrosis, accumulation of blood cells, displacement of the nucleus and completely damaged hepatic cells were noticed in pesticide-treated groups.

KEYWORDS: Alternations, Histopathology, Insecticide, Liver & Pearlspot

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Original Article

INTRODUCTION

Insecticidal products containing pyrethroids have been widely used to control insect pests in agriculture, public health, homes, and gardens (Amweg and Weston, 2005; Oros and Werner, 2005). Lambda-cyhalothrin(trade name Karate) is a pyrethroid insecticide. Lambda-cyhalothrin was first approved for use in the UK in 1988 (Advisory Committee on Pesticides, 1988). Various authors have also reported their effect on non-target organisms including fish (Campana *et al*., 1999; Radhakrishnan Nair, 2002; Ogueji and Auta, 2007; Velmurugan *et al*., 2007; Saravanan *et al*., 2009).

The study of structural damage of organs or tissues is an integral part of pollution toxicology. Histopathological alternations are biomarkers of the effect of exposure to environmental stressors, revealing prior alternations in physiological and/or biochemical function (Hinton *et al*., 1992). Tissue injuries and damages in organs can result in the reduced survival, growth and fitness, the low reproductive success or increase of susceptibility to pathological agents. Frequency and intensity of tissue lesions depend on the concentrations of insecticides and the length of the period fish are exposed to toxins. Nevertheless, many insecticides cause-specific or non-specific histopathological damage (Fanta *et al*., 2003). Fishes are good suitable bio-indicators of environmental pollution monitoring and can play significant roles in assessing potential risk associated with contamination in the aquatic environment since they are directly exposed to chemicals resulting from agricultural

production or indirectly through the food chain of the ecosystem (Lakra and Nagpure, 2009).

Sarkar *et al*. (2005) showed that the histopathological alternations in the gills and liver of *Labeorohita* (Hamilton) were due to the exposure of the fish to carbofuran and cypermethrin. Histopathological tissue changes, especially in the gills and liver in the rainbow trout exposed to cypermethrin was observed by Velisek*et al*. (2006). Altinok and Capkin (2007) studied that histopathology of rainbow trout exposed to sub-lethal concentrations of methiocarb and endosulfan. Dutta *et al*. (1993) studied malathion induced the histopathological alternations in the liver of freshwater catfish, *Heteropneustesfossilis* (Bloch).

The liver is a very important organ performing vital functions like detoxification, synthesis of several components of blood plasma, storage of glucose in the form of glycogen, and release of glucose. Pollutant related morphological, histological and histopathological alternations in the liver of fish have been studied by various scholars (Kendall, 1977; Dubale and Shah, 1979; Mandal and Kulshrestha, 1980; Kulshrestha and Jauhar, 1984). Liver plays a primary role in the metabolism and excretion of xenobiotic compounds with morphological alternations occurring in some toxic conditions (Rocha and Monteiro, 1999).

Histological studies on fish have revealed that various toxicants have produced pathological changes in the tissues such as macrobiotic changes in the liver, tubular damage of kidneys, gill and lamellar abnormalities (Ramalingam *et al*., 2000). Histopathological techniques are rapid, sensitive, reliable and comparatively inexpensive tools for the assessment of stress-response to pollutants. Hence the different sub-lethal effects of lambda-cyhalothrin on the morphology of liver tissues from *E. suratensis* were studied.

MATERIALS AND METHODS

A group of 10 fishes without sex determination were exposed to 0.005 ppm, 0.006, 0.008, 0.013 and 0.026 ppm (1/20, 1/16, 1/12, 1/8 and 1/4 sub-lethal concentration of LC_{50} value) for observing the histopathological changes. The test solution and the pesticide –free medium in which fishes were maintained, were renewed daily. Fishes were randomly selected from control groups and treated groups, for histopathological observations by sampling after 60 days of pesticide exposure. The liver of control and pesticide-treated *E. suratensis* were taken out and a histological study was carried out by employing the Culling (1974) method.

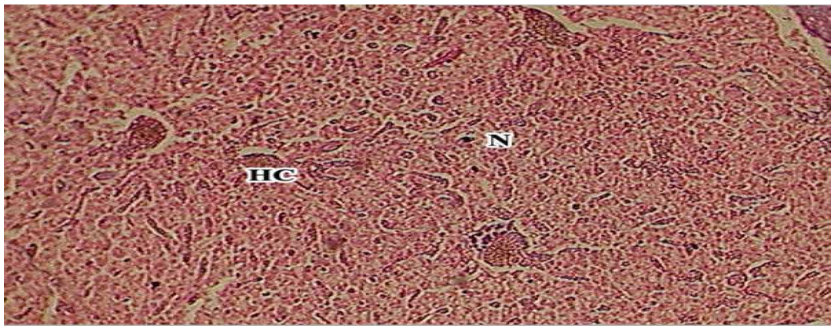
The dissected tissues were fixed in Bouin's fixative (saturated aqueous solution of picric acid (75 %), formalin (25 %), Acetic acid (5 %) for 24 hrs. The fixed tissues were washed in running tap water overnight. Then, the tissues were preserved in 50% isopropyl alcohol and then dehydrated in ascending grades of isopropyl alcohol. Finally, the tissues were completely dehydrated through two changes of 100 percent alcohol for 20-30 minutes each. The tissues were, then, dealcoholized by two changes of xylol, 20-30 minutes in each change. It was followed by infiltration in xylol saturated with paraffin wax of the congealing point 56-58ºC. The tissues were left in molten wax for at least 3o minutes in the embedding bath. In order to remove all the clearing agent (xylol), three changes in paraffin were made for infiltration. Then, they were embedded in labeled paper "boats" after the proper orientation of the processed tissues. The embedded tissues were sectioned at 7 µm (micrometer) thickness in a rotary microtome (WEXWAX-SPENCER). The ribbon of the section was cut into strips and floated on warm water poured over acid-cleaned glass slides pasted with a thin film of affixative. Mayer's albumen and Hampt's affixative were used as affixatives.

After complete air drying for a few hours, the slides were left overnight on a slide warmer whose temperature was maintained at 10-15º C below the melting point of the paraffin wax. Then the sections were deparaffinized in xylene and dehydrated in decreasing grades of isopropyl alcohol, 90%, 70%, and 50% alcohol series and finally in distilled water. The sections were stained with Ehrlich's haemotoxylin for 15 min and destained in dilute hydrochloric acid. After dehydrating in 50%, 70%, 90% isopropyl alcohol and distilled water, they were counter-stained in Eosin. The stained sections were rapidly dehydrated in ascending grades of isopropyl alcohol and counterstained in Eosin. The slides were cleared in xylene and mounted with DPX mountant. Selected slides were photographed by using a computerized Kyowa-Trinocular Microscope with CC TV attached.

RESULTS

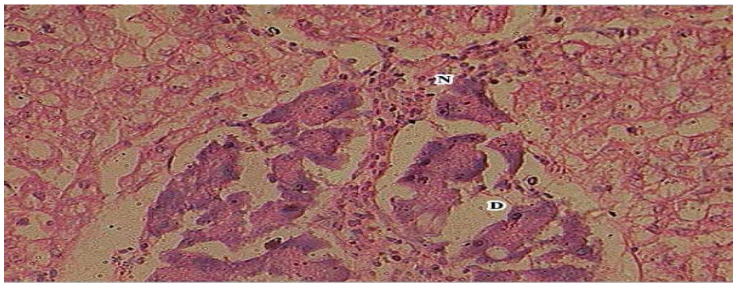
The various structural alterations were observed under a light microscope in the sections of liver tissues of fish from the treated group. The tissues of fishes from lambda-cyhalothrin-treated groups appeared in a structure different from those of the control group fishes. Liver of teleosts is a bilobed gland comprising of two tissue compartments, the parenchyma, and stroma. The parenchyma consists of hepatocytes and the stroma consist of hepatopancreas, bile duct, blood vessels, and connective tissue. The parenchymatous cells forming cords lie irregularly and get separated by blood sinusoids. Hepatocytes are polygonal cells with a prominent spherical central nucleus and densely stained nucleolus. Each sinusoid consists of an outer peripheral connective tissue and an inner lining of endothelial cells. In the control group, the liver exhibited a normal architecture with hepatocytes presenting a homogenous cytoplasm and a large central or sub-central spherical nucleus (Plate 1).

On chronic exposure of *E. suratensis* to lambda-cyhalothrin, the liver showed some interesting changes in the normal structure. The histological alternations like necrosis and degeneration of hepatocytes (Plate 2) were prominent changes observed in the liver of *E. suratensis* exposed to the lower concentration of lambda-cyhalothrin (0.005 ppm). When the fishes were exposed to 0.006 ppm and 0.008 ppm changes noticed in the liver include lymphatic aggregation, degeneration, necrosis in certain places, rupture of the hepatocytes and haemorrhage(Plate 3 and 4). The liver of *E. suratensis* exposed to the higher concentration of (0.013 ppm and 0.026 ppm) lambda-cyhalothrin revealed coagulative necrosis, rupture of hepatocytes, accumulation of blood cells, displacement of the nucleus and completely damaged hepatic cells were noticed (Plate 5 and 6).



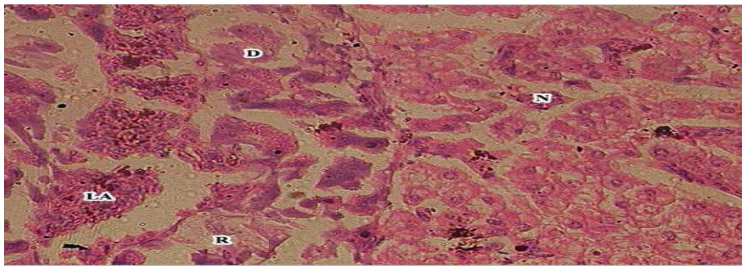
N-Nucleus HC-Hepatic Cell

Plate 1: Section showing the Liver of Control *E. Suratensis* (100X)



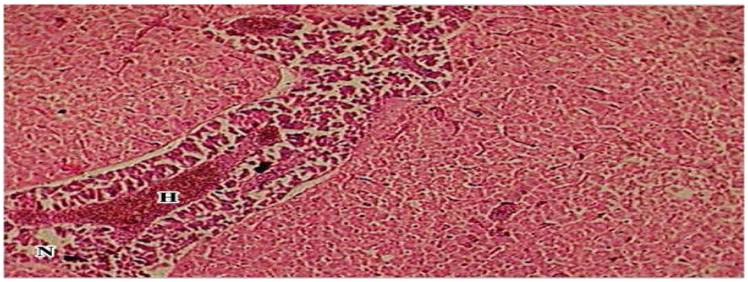
N-Necrosis D-Degeneration of Hepatocytes

Plate 2: Section showing the liver of *E. Suratensis* exposed to 0.005 ppm Concentration of λ-Cyhalothrin(400X)



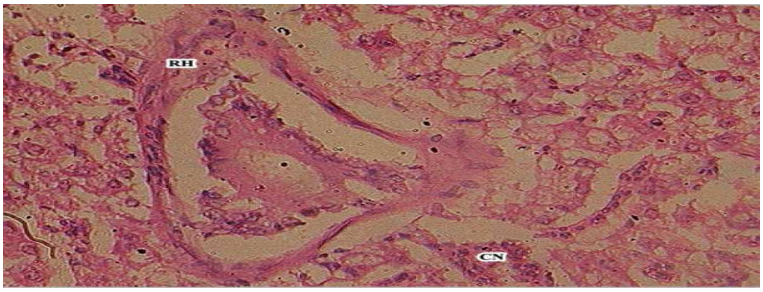
LA-Lymphatic Aggregation D-Degeneration N-NecrosisR-Rupture of Hepatocytes

Plate 3: Section showing the Liver of *E. Suratensis* Exposed to 0.006 ppm Concentration of λ -Cyhalothrin (400X)



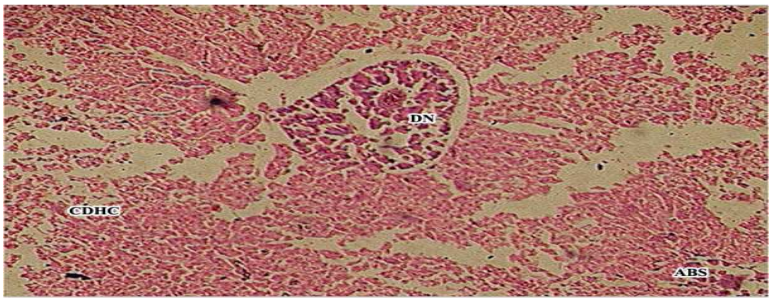
H-Haemorrage N-Necrosis

Plate 4: Section showing the Liver of *E. Suratensis* Exposed to 0.008ppm Concentration of λ-Cyhalothrin (200X)



CN- Coagulative Necrosis RH-Rupture of Hepatocytes

Plate 5: Section showing the Liver of *E. Suratensis* Exposed to 0.013 ppm Concentration of λ-Cyhalothrin (400X)



CDHC-Completely Damaged Hepatic Cells DN-Displacement of the Nucleus
ABS-Accumulation of Blood Cells in Sinusoids

Plate 6: Section showing the Liver of *E. Suratensis* Exposed to 0.026 ppm Concentration of λ-Cyhalothrin (200X)

DISCUSSIONS

The organ most associated with the detoxification and biotransformation process is the liver and due to its function, position and blood supply, it is also one of the organs most affected by contaminants in the water. The present results included many alterations in the liver such as necrosis, damaged hepatocytes, lymphatic aggregation, degeneration, rupture of the hepatocytes, haemorrhage, coagulative necrosis, accumulation of blood cells, displacement of the nucleus and completely damaged hepatic cells were noticed. These changes may be attributed to direct toxic effects of pollutants on hepatocytes since the liver is the site of detoxification of all types of toxins and chemicals (Soufy*et al*., 2007).

The vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the circulation system (Gingerich, 1982). And also, (Ologo *et al*., 2005) observed degeneration of the hepatocytes and focal necrosis in the liver of *Clariasgariepinus*exposed to lead. Anees (1978) reported necrosis and destruction of cytoplasmic material in a freshwater teleost *C. punctatus* exposed chronic levels of organophosphorus pesticides, dimethoate. Degenerative changes were also noticed in the liver of *C. Punctatus* in response to dieldrin, linolane and endrin intoxications (Mathur, 1976; Sastry and Sharma, 1978a). Kulshrestha and Jauhar (1984) reported splitting of tissue, rupture of the cell membrane, binucleate hepatocytes and disorganization of hepatic cords in *C. striatus* exposed to a sublethal dose of thiodan and sevin.

A number of pathological changes have been reported in fish exposed to different pesticides. Tilak *et al*. (2005) observed the similar changes in the liver of *Catlacatla* exposed to chlorpyrifos. The pathological changes included degeneration of cytoplasm in hepatocytes, atrophy, the formation of vacuoles, and rupture in blood vessels, necrosis and disappearance of hepatocyte cell membrane disposition. Hepatic cords are found to be decreased in size and nucleus became pyknotic. Radhaiah and JayanthaRao (1992) reported moderate cytoplasmic degeneration in hepatocytes, the formation of vacuoles, a rupture in blood vessels and appearance of blood vessels among hepatocytes and pyknotic nuclei in the hepatic tissue of *T. Mossambica* exposed to fenvalerate. the *et al.* (2005) found that exposing 7-day-old larvae of the fish *Sarcamentosplittail* to sub-lethal concentrations of esfenvalerate for one week induced vacuolar degeneration and cell necrosis in the liver. Histological changes in the liver of *T. mossambica* after exposure to the organophosphate monocrotophos were reported by Desai *et al*. (1984). At the initial stage of intoxication, necrosis and vacuolization of hepatocytes were recorded, while fatty degeneration was observed later on. Elezabi*et al.* (2001) studied the effect of malathion on the fish *Oreochromisniloticus*and their results showed that this insecticide-induced many histopathological changes in the liver and gills of the fishes. These changes were hemorrhage, necrosis, and destruction of lamellae of the

lungs, and necrosis and lipidosis in the liver. Sakr*et al*. (2001) studied the effect of the organophosphorus insecticide (Hostathion) on the liver of the catfish, *Clariasgariepinus*.

Studies on the toxicity of aldrin to *Cyprinuscarpio*liver indicated vacuolation in the hepatic cells (Ratnakar and Awasthy, 1979). The connective tissue has degenerated and some cells showed the rupture of the cell membrane. Degeneration of hepatocytes with extensive pycnosis and involution to carbofuran exposed *Channapunctatus.* This trend was similar to the present observations (Krishna Gopal and Ram, 1994). Necrosis of liver cells with swollen or disintegrating cells replaced by debris after selenium exposure has been reported in the literature (Srivastava and Srivastava, 1995).

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